Amendments to the Claims

This listing of claims will replace all prior versions, and listings, of claims in the application.

Listings of Claims

1. (withdrawn) A method of detecting a target sequence, comprising:

(a) contacting a first and second probe with a target sequence under conditions where complementary probes form a hybridization complex with said target sequence, said first probe comprising an upstream universal priming site and a target-specific sequence, said second probe comprising a downstream universal priming site and a target-specific sequence, wherein one of said first or second probes comprise an adapter sequence;

(b) extending said first or second probe of said hybridization complex to form a modified probe;

(c) amplifying said modified probe to form an amplicon, and

(d) detecting said amplicon.

2. (withdrawn) The method of claim 1, wherein step (b) further comprises ligating said modified probe to said first or second probe to form a ligated probe.

3. (withdrawn) The method of claim 1, wherein said amplifying said modified probe comprises exponential amplification.

4. (withdrawn) The method of claim 1, wherein said amplifying said modified probe comprises linear amplification.

5. (withdrawn) The method of claim 1, wherein said amplifying said modified probe comprises both exponential and linear amplification.

6. (withdrawn) The method of claim 1, wherein said first or second probe comprise more than one universal priming site.

7. (withdrawn) The method of claim 1, further comprising a plurality of first and second probes and a plurality of target sequences.

8. (withdrawn) The method of claim 1, wherein said amplicon comprises a label.

9. (withdrawn) The method of claim 1, further comprising removing non-hybridized probes.

10. (withdrawn) The method of claim 1, wherein said first and second probes further comprise a solid support.

11. (withdrawn) The method of claim 1, wherein said target sequence further comprises a solid support.

12. (withdrawn) The method of claim 1, further comprising contacting said adapter sequence of said first or second probe, or an amplicon thereof, with a capture probe to form a hybridization complex comprising said adapter sequence and said capture probe.

13. (withdrawn) The method of claim 1, further comprising producing a report of a detected target sequence.

14. (withdrawn) A method of detecting the relative amounts of two or more target sequences, comprising:

(a) contacting a first and a second probe with first and second target sequences in an initial population under conditions where complementary probes form a hybridization complex with said target sequences, said first and second probes comprising a universal priming site, an adapter sequence and a target-specific sequence;

(b) linearly amplifying said first and second probes forming said hybridization complex to produce first and second amplicons having distinctive adapter sequences, and

(c) determining a relative amount of said first and second amplicons distinguishable by said adapter sequence, wherein said relative amount of said amplicons is indicative of the relative amounts of said first and second target sequences in said initial population.

15. (withdrawn) The method of claim 14, wherein said universal priming site comprises an RNA polymerase priming site.

16. (withdrawn) The method of claim 15, wherein said RNA polymerase priming site comprises a RNA polymerase promoter sequence corresponding to T7, T4, T3, SP6, RNA polymerase from Thermus, species or Q beta replicase from bacteriophage.

17. (withdrawn) The method of claim 15, wherein said step of linearly amplifying comprises in vitro transcription (IVT).

18. (withdrawn) The method of claim 14, wherein said first and second amplicons comprise a label.

19. (withdrawn) The method of claim 18, wherein said first and second amplicons comprise different labels.

20. (withdrawn) The method of claim 14, further comprising removing non-hybridized first and second probes.

21. (withdrawn) The method of claim 14, wherein said first or second probes further comprise a solid support.

22. (withdrawn) The method of claim 14, wherein said first and second target sequences further comprise a solid support.

23. (withdrawn) The method of claim 14, further comprising contacting said adapter sequence of said first or second probe, or an amplicon thereof, with a capture probe to form a hybridization complex comprising said adapter sequence and said capture probe.

24. (withdrawn) The method of claim 14, further comprising modifying said first and second probes.

25. (withdrawn) The method of claim 14, wherein each of said first and second probes further comprise a first and second pair of probes.

26. (withdrawn) The method of claim 25, further comprising modifying said first and second pair of probes.

27. (withdrawn) The method of claim 26, wherein said modifying comprises polymerase extension, ligation or both.

28. (withdrawn) The method of claim 26, further comprising modifying a first probe from each of said first and second pair of probes by polymerase extension to form an extended probe, and ligating said extended probe to a second probe from each of said first and second pair of probes.

29. (withdrawn) The method of claim 14 or 25, further comprising contacting a of plurality probes with a plurality of target sequences to form a plurality of hybridization complexes, each of said plurality comprising more than two.

30. (withdrawn) The method of claim 26, wherein said plurality of probes comprises at least 8, 10 96, 192, 384, 1152or1536.

31. (withdrawn) The method of claim 14 or 25, wherein said first and second probes further comprise a second universal priming site.

32. (withdrawn) The method of claim 31, further comprising a step of exponential amplification.

33. (withdrawn) The method of claim 14 or 32, further comprising hybridizing a blocking primer to suppress amplicon production by linear or exponential amplification, wherein said suppression lowers the dynamic range of an amplicon signal.

34. (withdrawn) The method of claim 14, further comprising producing a report of said relative amounts of two or more detected target sequences.

35. (currently amended) A method of detecting the relative amounts of two or more target sequences, comprising:

(a) hybridizing a first and second pair of ligation probes with first and second target sequences in an initial population to form first and second ligation complexes, said first and second pair of ligation probes each comprising a first target-specific sequence of a first probe, a second target-specific sequence of a second probe, a universal priming site and an adapter

sequence that differs from said first and second target sequences, said ligation complexes capable of being ligated when said target sequences are complementary to said first and second probes;

(b) ligating said first and second ligation complexes to form first and second ligated probes;

(c) linearly amplifying said first and second ligated probes to produce first and second amplicons, and

(d) determining a relative amount of said first and second amplicons, wherein said relative amount of said amplicons is indicative of ~~the~~ relative amounts of said first target sequence relative to said ~~and~~ second target sequences in said initial population.

36. (original) The method of claim 35, wherein said universal priming site comprises a RNA polymerase priming site.

37. (original) The method of claim 36, wherein said RNA polymerase priming site comprises a RNA polymerase promoter sequence corresponding to T7, T4, T3, SP6, RNA polymerase from Thermus species or Q beta replicase from bacteriophage.

38. (original) The method of claim 36, wherein said step of linearly amplifying comprises in vitro transcription (WVT).

39. (original) The method of claim 35, wherein said first and second amplicons comprise a label.

40. (original) The method of claim 39, wherein said first and second amplicons comprise different labels.

41. (original) The method of claim 35, further comprising removing non-hybridized first or second probes.

42. (original) The method of claim 35, wherein said first or second probes of said pair of ligation probes further comprise a solid support.

43. (original) The method of claim 35, wherein said first or second target sequences further comprise a solid support.

44. (original) The method of claim 35, further comprising contacting said adapter sequence of said first or second ligated probe, or an amplicon thereof, with a capture probe to form a hybridization complex comprising said adapter sequence and said capture probe.

45. (original) The method of claim 35, further comprising modifying said first and second pair of ligation probes.

46. (original) The method of claim 45, wherein said modifying comprises polymerase extension.

47. (original) The method of claim 35 or 45, further comprising contacting a plurality of pairs of ligation probes with a plurality of target sequences to form a plurality of ligation complexes, each of said plurality comprising more than two.

48. (currently amended) The method of claim 47, wherein said plurality of pairs of probes comprises at least 8, 96, 192, 384, 1152 or 1536.

49. (original) The method of claim 35, wherein said first or second probes further comprise a second universal priming site.

50. (original) The method of claim 49, further comprising a step of exponential amplification.

51. (original) The method of claim 35 or 50, further comprising hybridizing a blocking primer to suppress amplicon production by linear or exponential amplification, wherein said suppression lowers the dynamic range of an amplicon signal.

52. (original) The method of claim 35, further comprising producing a report of said relative amounts of two or more detected target sequences.

53. (withdrawn) A method of amplifying a target sequence to produce a signal within a dynamic range of a detection assay, comprising:

(a) hybridizing a target-specific probe having an upstream universal priming site (WUP), a downstream universal priming site (DUP) and an adapter sequence with a set of differential primers, said set of differential primers comprising an upstream primer and first and

second downstream primers, said second downstream primer having a lower Tm compared to said upstream primer and said first downstream primer;

(b) amplifying said probe with said set of differential primers for two or more cycles of enzymatic polymerization;

(c) increasing hybridization stringency to suppress hybridization of said second downstream primer, and

(d) amplifying said probe from said upstream and said first downstream primers of said set for at least one cycle of enzymatic polymerization, wherein differential signals of amplicons produced from amplification of said first or said second downstream primers fall within a dynamic range of a detection assay.

54. (withdrawn) The method of claim 53, further comprising detecting said differential signals produced from said amplicons.

55. (withdrawn) The method of claim 53, wherein said signal produced from amplicons of said first downstream primer produces a signal within said dynamic range for a low abundant target sequence.

56. (withdrawn) The method of claim 53, wherein said signal produced from amplicons of said second downstream primer produces a signal within said dynamic range for an abundant target sequence.

57. (withdrawn) The method of claim 53, wherein said set of differential primers further comprise a plurality of second downstream primers, each primer of said plurality of second downstream primers comprising a different Tm compared to said first downstream primer.

58. (withdrawn) The method of claim 57, wherein said first downstream primer and said plurality of second downstream primers result in said signal produced from amplicons within a low, medium or high dynamic range.

59. (withdrawn) The method of claim 53, wherein said probe is obtained by a method comprising contacting a probe with a target sequence under conditions where a complementary probe forms a hybridization complex with said target sequence, said probe

comprising an upstream universal priming site, a downstream universal priming site, an adapter sequence and a target-specific sequence.

60. (withdrawn) The method of claim 53, wherein said probe is obtained by a method comprising:

(a) contacting a first and second probe with a target sequence under conditions where complementary probes form a hybridization complex with said target sequence, said first probe comprising an upstream universal priming site and a target-specific sequence, said second probe comprising a downstream universal priming site and a target-specific sequence, wherein one of said first or second probes comprise an adapter sequence, and

(b) extending said first or second probe of said hybridization complex.

61. (withdrawn) The method of claim 60, further comprising ligating said first and second probes.

62. (withdrawn) The method of claim 53, wherein said probe is obtained by a method, comprising:

(a) hybridizing a pair of ligation probes with target sequence to form a ligation complex, said pair of ligation probes comprising a first target-specific sequence of a first probe, a second target-specific sequence of a second probe, an upstream universal priming site, a downstream universal priming site and an adapter sequence, said ligation complex capable of being ligated when said target sequences are complementary to said pair of ligation probes, and

(b) ligating said ligation complex to form a ligation probe.

63. (withdrawn) The method of claim 53, further comprising hybridizing a blocking primer to suppress amplification, wherein said suppression lowers the dynamic range of a signal produced from an amplicon thereof.

64. (withdrawn) The method of claim 63, wherein said blocking primer comprises a sequence lacking a universal priming site, contains a terminal dideoxy nucleotide or a non-ligatable chemical moiety.

65. (withdrawn) The method of claim 53, further comprising a plurality of target-specific probes and a plurality of differential primer sets.

66. (withdrawn) The method of claim 53, wherein said amplicons comprise a label.

67. (withdrawn) The method of claim 53, wherein said amplicons produced from said first and second downstream primers comprise different labels.

68. (withdrawn) The method of claims 69, 60, or 62, further comprising removing non-hybridized probe.

69. (withdrawn) The method of claim 53, wherein said probe further comprises a solid support.

70. (withdrawn) The method of claim 53, wherein said target sequence further comprises a solid support.

71. (withdrawn) The method of claim 53, further comprising contacting said adapter sequence of said probe, or an amplicon thereof, with a capture probe to form a hybridization complex comprising said adapter sequence and said capture probe.

72. (withdrawn) The method of claims 53, 59, 60 or 62, further comprising a step of linear amplification.

73. (withdrawn) The method of claim 53, further comprising producing a report of a detected target sequence.

74. (withdrawn) A method of amplifying a target sequence to produce a signal within a dynamic range of a detection assay, comprising:

(a) hybridizing a target-specific probe having an upstream universal priming site (UUP), a downstream universal priming site (DUP) and an adapter sequence with a set of differentially labeled primers, said set of differentially labeled primers comprising an upstream primer and a differentially labeled downstream primer, said differentially labeled downstream primer comprising two different labels corresponding to different relative primer concentrations, and

(b) amplifying said probe with said set of differentially labeled primers for two or more cycles of enzymatic polymerization, wherein differential signals of amplicons produced

from amplification of said probe with said first or said second downstream primers fall within a dynamic range of a detection assay.

75. (withdrawn) The method of claim 74, further comprising detecting said differential signals produced from said amplicons.

76. (withdrawn) The method of claim 74, wherein said signal produced from amplicons of a downstream primer present at a higher relative concentration produces a signal within said dynamic range for a low abundant target sequence.

77. (withdrawn) The method of claim 74, wherein said signal produced from amplicons of a downstream primer present at a lower relative concentration produces a signal within said dynamic range for an abundant target sequence.

78. (withdrawn) The method of claim 74, wherein said probe is obtained by a method comprising contacting a probe with a target sequence under conditions where a complementary probe forms a hybridization complex with said target sequence, said probe comprising an upstream universal priming site, a downstream universal priming site, an adapter sequence and a target-specific sequence.

79. (withdrawn) The method of claim 74, wherein said probe is obtained by a method comprising:

(a) contacting a first and second probe with a target sequence under conditions where a complementary probes form a hybridization complex with said target sequence, said first probe comprising an upstream universal priming site and a target-specific sequence, said second probe comprising a downstream universal priming site and a target-specific sequence, wherein one of said first or second probes comprise an adapter sequence, and

(b) extending said first or second hybridization complex.

80. (withdrawn) The method of claim 79, further comprising ligating said first and second probes.

81. (withdrawn) The method of claim 74, wherein said probe is obtained by a method, comprising:

(a) hybridizing a pair of ligation probes with target sequence to form a ligation complex, said pair of ligation probes comprising a first target-specific sequence of a first probe, a second target-specific sequence of a second probe, an upstream universal priming site, a downstream universal priming site and an adapter sequence, said ligation complex capable of being ligated when said target sequences are complementary to said pair of ligation probes, and

(b) ligating said ligation complex to form a ligation probe.

82. (withdrawn) The method of claim 74, further comprising a plurality of target-specific probes and a plurality of differentially labeled primer sets.

83. (withdrawn) The method of claim 74, wherein said probe further comprises a solid support.

84. (withdrawn) The method of claim 74, wherein said target sequence further comprises a solid support.

85. (withdrawn) The method of claim 74, further comprising contacting said adapter sequence of said probe, or an amplicon thereof, with a capture probe to form a hybridization complex comprising said adapter sequence and said capture probe.

86. (withdrawn) The method of claim 74, further comprising a step of linear amplification.

87. (withdrawn) The method of claim 74, further comprising producing a report of a detected target sequence.

88. (withdrawn) A method of amplifying a target sequence to produce a signal within a dynamic range of a detection assay, comprising:

(a) hybridizing a target-specific probe having at least one universal priming site and an adapter sequence with at least one primer, and

(b) amplifying said probe from said at least one primer in the presence of at least two differently labeled nucleotides corresponding to different relative label concentrations, wherein differential signals of amplicons produced from said differently labeled nucleotides fall within a dynamic range of a detection assay.

89. (withdrawn) The method of claim 88, further comprising detecting said differential signals produced from said amplicons.

90. (withdrawn) The method of claim 88, wherein said signal produced from amplicons of a labeled nucleotide present at a higher relative concentration produces a signal within said dynamic range for a low abundant target sequence.

91. (withdrawn) The method of claim 88, wherein said signal produced from amplicons of a labeled nucleotide present at a lower relative concentration produces a signal within said dynamic range for an abundant target sequence.

92. (withdrawn) The method of claim 88, wherein said probe is obtained by a method comprising contacting a probe with a target sequence under conditions where a complementary probe forms a hybridization complex with said target sequence, said probe comprising an upstream universal priming site, a downstream universal priming site, an adapter sequence and a target-specific sequence.

93. (withdrawn) The method of claim 88, wherein said probe is obtained by a method comprising:

(a) contacting a first and second probe with a target sequence under conditions where a complementary probes forms a hybridization complex with said target sequence, said first probe comprising an upstream universal priming site and a target specific sequence, said second probe comprising a downstream universal priming site and a target-specific sequence, wherein one of said first or second probes comprise an adapter sequence, and

(b) extending said first or second hybridization complex.

94. (withdrawn) The method of claim 93, further comprising ligating said first and second probes.

95. (withdrawn) The method of claim 88, wherein said probe is obtained by a method, comprising:

(a) hybridizing a pair of ligation probes with target sequence to form a ligation complex, said pair of ligation probes comprising a first target-specific sequence of a first probe, a second target-specific sequence of a second probe, an upstream universal priming site, a

downstream universal priming site and an adapter sequence, said ligation complex capable of being ligated when said target sequences are complementary to said pair of ligation probes, and

(b) ligating said ligation complex to form a ligation probe.

96. (withdrawn) The method of claim 88, further comprising a plurality of target sequence probe complexes and a plurality of differentially labeled primer sets.

97. (withdrawn) The method of claim 88, further comprising linear amplification.

98. (withdrawn) The method of claim 88, further comprising exponential amplification.

99. (withdrawn) The method of claim 88, further comprising producing a report of a detected target sequence.

100. (withdrawn) A plurality of target sequence detection components, comprising a plurality of probes, each of said probes comprising a target-specific sequence, a universal priming site and an adapter sequence, and information correlating said target-specific sequence with an associated adapter sequence.

101. (withdrawn) The target sequence detection components of claim 100, wherein said plurality of probes comprises 96, 192, 384, 1152, or 1536 target-specific probes.

102. (withdrawn) The target sequence detection components of claim 100, further comprising ancillary reagents.

103. (withdrawn) The target sequence detection components of claim 102, wherein said ancillary reagents are selected from the group consisting of buffer, extension buffer, probe hybridization buffer, amplification buffer, ligation buffer, wash buffer, instructions for making a buffer, biotin, nucleotides, DNA polymerase, RNA polymerase, ligase, a plurality of microspheres and a plurality of magnetic beads.